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Genotype x environment effect on starch functionality of field pea and fababean varieties

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Effect of Genotype and Environment and their Interaction on the Concentration of Starch and Protein in, and the Physicochemical Properties of Starch from, Field Pea and Fababean (Project #20070153)

Final Report

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Table of Contents

List of Tables

List of Tables (Cont'd)

List of Figures

List of Abbreviations

ΔH – Endothermic enthalpy of gelatinization

CV – Cooling viscosity

DenDF – Denominator degrees of freedom

FV – Final viscosity

Ge – Genotype

Loc – Location

NumDF – Numerator degrees of freedom

PT – Pasting temperature

PV – Pasting viscosity

Tc – Completion temperature of gelatinization

To – Onset temperature of gelatinization

Tp – Peak temperature of gelatinization

TV – Trough viscosity

Executive Summary

The effects of genotype and environment, and their interaction, on the concentrations of starch and protein in, and the physicochemical properties (amylose concentration and thermal and pasting characteristics) of starch from, pea and fababean were investigated. Samples of ten pea genotypes were obtained from material grown in four locations [Elrose, Hodgeville, Melfort, Saskatoon (Sutherland)] in each of 2006 and 2007. Samples of eleven fababean genotypes were obtained from material grown in three locations in 2006 [Melfort, Saskatoon (Preston), Saskatoon (SPG)] and 2007 [Outlook, Saskatoon (Preston), Roblin].

Genotypic differences in the concentrations of starch and protein in pea and fababean were observed. On a dry weight basis, pea and fababean contained 44.1-46.2% and 41.1-47.5% starch and 24.2-27.5% and 27.5-32.4% protein, respectively. Genotypic differences in the onset temperature (To) and peak temperature (Tp) of gelatinization of fababean starch, and in the pasting, trough, cooling and final viscosities of pea starch and fababean starch, also were observed. Significant two-way interactions (location x genotype) were observed for the concentration of starch in fababean and the amylose concentration in, and the To, endothermic enthalpy of gelatinization (ΔH) and trough viscosity of, fababean starch. Significant three-way interactions (location x year x genotype) were observed for the concentration of starch in pea and the pasting, trough, cooling and final viscosities of pea starch.

It was concluded that the differences observed in the concentrations of starch and protein in pea and fababean were sufficient to be of practical significance to end-users, but that the relatively small differences in the concentration of amylose in, and the physicochemical characteristics of, starch from pea and fababean were not. Some of the significant interactions observed, including the genotype by location interactions for protein concentration in pea and starch concentration in fababean, and the genotype by location by year interaction for starch concentration in pea, would complicate the manipulation through breeding of the concentrations of protein and starch in pea and fababean.

1.0 Introduction

1.1 Rationale, hypothesis and objectives

Research has shown that starches from different genotypes of wheat (Wootton and Mahdar 1993), rye (Gudmundsson and Eliasson 1991), maize (Yun and Matheson 1993), millet (Yanez et al. 1991), cassava (Asaoka et al. 1991) and lentil (Hoover and Manuel 1995) differ in functionality. However, a study of four pea genotypes detected minimal differences in starch functionality (Ratnayake et al. 2001). A subsequent study of genetic markers in pea genotypes (Tar'an et al. 2005) found that the genotypes used by Ratnayake et al. (2001) were closely related. A broader study on the effects of genotype and the environment, and their interaction, on starch functionality in pea and fababean may provide valuable information to pea and fababean breeders and processors. The primary objectives of this research were: i) to determine whether differences existed among pea and fababean genotypes with respect to their starch and protein concentrations and with respect to the concentration of amylose in, and the thermal and pasting properties of, starch isolated from pea and fababean; and ii) to determine the impact of the environment on the concentrations of starch and protein in pea and fababean, and on the concentration of amylose in, and the thermal and pasting properties of, starch isolated from pea and fababean.

1.2 Genotypic differences in starch functionality

The functionality of wheat (Wootton and Mahdar 1993), rye (Gudmundsson and Eliasson 1991), maize (Yun and Matheson 1993), millet (Yanez et al.1991) and cassava (Asaoka et al. 1991) starches varies with genotype. Wootton and Mahdar (1993) reported differences among wheat genotypes in starch gelatinization characteristics, granule crystallinity and molecular weight. The enthalpies of gelatinization of twenty-one Australian wheat genotypes exhibited significant differences and were correlated with amylopectin content and wheat hardness (Wootton and Mahdar 1993). Millet genotypes displayed different amylose concentrations and thermal and pasting properties (Yanez et al.1991).

1.3 Genotypic differences among legume starches

Hoover and Ratnayake (2002) investigated starch from three black bean genotypes and reported significant differences in swelling factor (determined by a colourimetric method using blue dextran), amylose leaching, onset (To) and peak (Tp) gelatinization temperatures, and endothermic enthalpy of gelatinization (ΔH). Additionally, these authors investigated two genotypes of each of chickpea, lentil, navy bean, pea and pinto bean, and reported minimal starch-related differences between genotypes. Differences in swelling factor were detected between the two chickpea and the two pea genotypes, in To between the two pea and the two lentil genotypes, and in ΔH between the two pea, the two chickpea and the two pinto bean genotypes. Hoover and Manuel (1995) investigated physicochemical differences between starches from Laird and CDC Gold lentil, and reported differences in amylose-lipid complexation, swelling factor, enzyme and acid hydrolysis rates, pasting and gelatinization properties, and retrogradation. Swelling factor, pasting temperature, and enzymatic and acid hydrolysis of starch from CDC Gold lentil were greater than those of starch from Laird lentil. Amylose leaching, pasting viscosities, To, completion temperature of gelatinization (Tc) and ΔH were greater in starch from Laird lentil than in CDC Gold lentil starch. Two genotypes of grass pea (*Lathyrus sativus*), NC8A97 and Lath 96, varied in B-polymorph content, swelling, amylose leaching, peak viscosity, setback, shear stability and the extent of enzyme and acid hydrolysis (Jayakody et al. 2007).

Ratnayake et al. (2001) reported no significant differences among starches from four pea genotypes (Carneval, Carrera, Grande and Keoma) in swelling factor, pasting temperature, freeze-thaw characteristics or the rate of acid and enzyme hydrolysis, despite variations in B-polymorph content and amylopectin branching. Carneval had a higher To (by 0.4°C) than did Carrera, Grande and Keoma. Grande had a higher Tp than did Carneval, Carrera and Keoma (67.5 versus 67.0, 66.8 and 67.0°C). Carrera had a lower Tc (by 1°C) than Carneval, Grande and Keoma. The range in ΔH for these four genotypes was 11.2-11.5 J/g. The genotypes Carneval, Carrera, Grande and Keoma contained similar genetic markers and are closely related as compared to other pea genotypes available (Tar'an et al. 2005).

Czuchajowska et al. (1998) investigated two pea genotypes, Latah and SS Alaska, and reported no differences in To, Tp, ΔH, swelling power (determined by a gravimetric method), gel hardness at 22°C, gel cohesiveness or gel springiness.

Tulbek and Simsek (2007) analyzed the starch concentrations in pea genotypes (Miami, Nitouche, DS Admiral, Eclipse, Majoret, Cruiser and CDC Mozart) grown in North Dakota and found that starch concentration differed among genotypes. Miami had the highest starch concentration at 43.9%, and Majoret had the lowest at 40.9%. Simsek and Tulbek (2007) reported differences in enthalpy of retrogradation among pea genotypes (Miami, Nitouche, DS Admiral, Eclipse, Majoret, Cruiser and CDC Mozart), and each genotype produced a different pasting profile.

Chung et al. (2008) studied flours from yellow pea, lentil and chickpea that were grown in the same location in Saskatchewan in 2005. Flour from the yellow pea genotype 1329-12 differed from those from the yellow pea genotypes 1674-13 and 1215-33 in apparent amylose concentration, and flour from 1215-33 differed from those from 1329-12 and 1674-13 in total starch concentration. These researchers also reported significant differences between the lentil genotypes CDC Meteor and CDC Robin in apparent amylose and protein concentration, but not in total starch, free lipid or bound lipid concentration. Flour from the chickpea genotype Myles was significantly lower in total starch, free lipid and bound lipid concentration, and higher in protein concentration, than were flours from the other chickpea genotypes tested. Flour from yellow pea genotype 1329-12 was significantly lower in swelling power at 90°C than were flours from 1674-13 and 1215-33, but the genotypes were similar with respect to amylose leaching. Flours from the pea genotypes did not differ in To, Tp or Tc. Flours from the lentil genotypes CDC Meteor and CDC Robin differed in To and ΔH, but Tp and Tc were similar. Flours from the pea genotypes differed significantly in final viscosity, but otherwise exhibited similar pasting temperatures.

1.4 Differences in starch content and functionality due to environment

Four genotypes of cassava grown and harvested under different conditions exhibited differences in the texture of their starch gels. Differences within one genotype grown under different conditions also

were detected (Asaoka et al. 1991). These authors reported no differences in the crystallinity of the cassava starch granule due to genotype or time of harvest.

Cottrell et al. (1995) investigated starch from potatoes grown under three conditions: field, unheated glasshouse and heated glasshouse, and reported that warmer growing conditions increased the gelatinization temperature, amylose concentration and alpha-amylase resistance of potato starch.

Nikolopoulou et al. (2007) reported differences in the starch concentrations of three pea genotypes (FCPI, Fytorio and Palamas) grown in three locations over two years. These authors also reported that pea genotypes grown under conditions of less rainfall possessed lower starch and higher protein concentrations, and reported significance for location and the location by year interaction for differences in starch concentration.

2.0 Materials and Methods

The Crop Development Centre, University of Saskatchewan, provided samples of pea and fababean genotypes. Samples of ten genotypes of pea (Bluebird, CanStar, CDC Striker, CDC Tucker, Cooper, Cutlass, Fusion, Reward, SW Marquee, Tamora) grown in four locations [Elrose, Hodgeville, Melfort and Saskatoon (Sutherland)] in each of 2006 and 2007 were analyzed for their starch and protein concentrations, and the concentration of amylose in, and the thermal and pasting properties of, starch isolated from the ten genotypes. Samples from eleven fababean genotypes (CDC Fatima, CEB04928, Disco, Dixie, Gloria, NPZ3-7080, NPZ4-7460, NPZ4-7540, NPZ5-7530, Snowbird, SSNS-1) grown in three locations in 2006 [Melfort, Saskatoon (Sutherland), Saskatoon (SPG)] and 2007 [Outlook, Roblin and Saskatoon (Preston)] were analyzed for their starch and protein concentrations, and the concentration of amylose in, and the thermal and pasting properties of, starch isolated from the eleven genotypes. Due to crop loss or lack of sufficient seed for starch isolation, some fababean samples were missing, i.e. both replications from the Saskatoon (Preston) location, year 2006, genotype CEB04928; the Saskatoon (SPG) location, year 2006, genotypes Disco and Dixie; and one replication from the Saskatoon (SPG) location, year 2006, genotype NPZ3-7080. Starch and protein concentrations were determined on flour samples

ground using a cyclone mill (UDY Corp., Fort Collins, CO) to pass a 0.5-mm screen. The starch and protein concentrations in seed and the amylose concentration in starch were determined according to Holm et al. (1986), AACC method 46-30 (2000) and Demeke et al. (1999), respectively. A Rapid Visco Analyzer (Newport Scientific Ltd., Warriewood, Australia) was used to determine pasting properties according standard method #2 with an 8% slurry and a pH of 7.0. Thermal properties were determined by differential scanning calorimetry (TA Instruments, New Castle, DE) according to Ratnayake et al. (2001) with a starch sample size of 2 mg (db) and 6.4 µL of deionized water. Analyses were replicated a minimum of two times.

The procedure used to isolate starch from pea and fababean began by soaking 100 g of seed overnight in 500 mL of 0.5% aqueous ethanol (to inhibit microbial growth). The seed was then drained, rinsed and ground using a blender at low speed for 2 minutes. The puree was screened through 60- and 200-mesh sieves. The unders were centrifuged at 15,000 x *g* for 10 minutes and the supernatant was decanted and the protein scraped off the starch pellet. The starch pellet was reslurried in 0.05 N sodium hydroxide, mixed for 60 minutes and then filtered. This filter cake was reslurried in deionized water and the pH was adjusted to 7.0 using 0.5% N HCl and then filtered. This filter cake was reslurried in 50% aqueous ethanol, mixed for 5 minutes and then filtered. This filter cake was rinsed with 95% ethanol and dried overnight at room temperature.

SAS PROC MIXED (SAS Institute Inc, Cary, NC) was used to determine if starch and protein concentrations in seed and the amylose concentration in, and the thermal and pasting characteristics of, starch differed among genotypes and environments. Genotype was considered a fixed effect and year, location and replications within year by location were considered random effects. F-values were approximated as recommended by Satterthwaite (1946) [cited by Cochran and Cox (1992)]. The model ANOVA table and mean square expectations are presented in Table 2.1. Means were differentiated using Least Squares Means.

Table 2.1 General form of the ANOVA and mean square expectations.

Source of Variation	df[a]	Mean Squares Expectations[b]	Mean Square	F-Test
Year (Y)	(Y-1)	$\sigma^2_e + r\sigma^2_{gyl} + rl\sigma^2_{gy} + g\sigma^2_r + gr\sigma^2_{yl} + grl\sigma^2_y$	M1	M1/(M3+M6-M8)
Location (L)	(L-1)	$\sigma^2_e + r\sigma^2_{gyl} + ry\sigma^2_{gl} + g\sigma^2_r + gr\sigma^2_{yl} + gry\sigma^2_l$	M2	M2/(M3+M7-M8)
Year x Location	(Y-1)(L-1)	$\sigma^2_e + r\sigma^2_{gyl} + g\sigma^2_r + gr\sigma^2_{yl}$	M3	M3/(M4+M8-M9)
Replication (Y L)	YL(R-1)	$\sigma^2_e + c\sigma^2_r$	M4	M4/M9
Genotype (G)	(G-1)	$\sigma^2_e + r\sigma^2_{gyl} + rl\sigma^2_{cy} + ry\sigma^2_{gl} + Q(c)$	M5	(M5+M8)/(M6+M7)
Genotype x Year	(G-1)(Y-1)	$\sigma^2_e + r\sigma^2_{gyl} + rl\sigma^2_{gy}$	M6	M6/M8
Genotype x Location	(G-1)(L-1)	$\sigma^2_e + r\sigma^2_{gyl} + ry\sigma^2_{gl}$	M7	M7/M8
Genotype x Year x Location	(G-1)(Y-1)(L-1)	$\sigma^2_e + r\sigma^2_{gyl}$	M8	M8/M9
Error	YL(G-1)(R-1)	σ^2_e	M9	

[a] where y, l, r and g are number of years, locations, replications and genotypes, respectively

[b] σ^2_e = error of variance; σ^2_{gyl} = cultivar x year x location variance; σ^2_{gl} = cultivar x location variance; σ^2_{gy} = genotype x year variance; and Q(c) = quadratic function of genotype

[c] The F test ratio was approximated according to Satterthwaite (1946), as cited by Cochran and Cox (1992)

3.0 Results

3.1 Pea

Mean values from protein, starch and amylose analyses are presented in Table 3.1. CDC Striker and CDC Tucker contained the highest protein concentrations of the genotypes tested at 27.5 and 27.1% (dry basis), respectively. CDC Striker, CDC Tucker, Cutlass and Tamora had the lowest starch concentrations at 44.0, 44.1, 45.0 and 44.9% (dry basis), respectively. The amylose concentrations in starches from the ten genotypes ranged from 29.8-31.6%.

Comparison of the protein concentrations in the ten pea genotypes grown in four locations in 2006 and 2007 revealed a statistically significant interaction between location and genotype (Table 3.2). The location by year by genotype interaction for starch concentration also was significant. The dependent variables tested were protein, starch and amylose concentration, temperature of onset of gelatinization (To), peak gelatinization temperature (Tp), completion temperature of gelatinization (Tc), the difference between Tc and To (Tc-To), endothermic enthalpy of gelatinization (ΔH), pasting temperature (PT), pasting viscosity (PV), trough viscosity (TV), cooling viscosity (CV), and final viscosity (FV). The residual term (error term) was significant for all dependent variables tested, thus there were factors that had significant effects on the variables tested but that were not investigated in this study. A number of covariance parameters were found to be zero, indicating that these parameters did not influence the dependent variables.

An example of a Differential Scanning Calorimetry (DSC) scan of starch isolated from pea is presented in Figure 3.1. The ranges of means for To, Tp, Tc, ΔH and Tc-To were 61.7-64.1°C, 68.6-69.7°C, 87.0-88.0°C, 14.1-14.9 J/g and 23.2-24.7 °C, respectively (Table 3.3). Gentotype did not influence To, Tp, Tc, ΔH or Tc-To. A significant location by genotype interaction was detected for Tp (Table 3.4).

An example of a Rapid Visco Analyzer graph of starch isolated from pea is presented in Figure 3.2. Mean values for the pasting properties of starches from the ten pea genotypes are presented in Table 3.5. Pasting temperature means ranged from 74.1-75.1°C. Genotype, location or year did not influence PT

Table 3.1 Mean values for protein and starch concentrations (%, dry basis) in, and amylose concentrations (%) in starch isolated from, ten pea genotypes grown in four locations in each of 2006 and 2007.*

Genotype	Protein	Starch	Amylose
Bluebird	25.0 ± 1.2a	45.5 ± 2.4a	31.5 ± 1.7a
CanStar	24.2 ± 1.2a	46.0 ± 1.5a	30.5 ± 1.0a
CDC Striker	27.5 ± 1.0b	44.0 ± 1.4b	31.1 ± 1.0a
CDC Tucker	27.1 ± 1.1b	44.1 ±1.4b	30.6 ± 1.4a
Cooper	25.5 ± 1.4a	45.2 ± 1.7a	31.6 ± 1.6a
Cutlass	25.0 ± 1.8a	45.0 ± 0.7ab	30.9 ± 1.5a
Fusion	24.4 ± 1.1a	45.2 ± 1.3a	29.8 ± 1.4a
Reward	25.5 ± 1.5a	46.2 ± 1.7a	30.9 ± 1.9a
SW Marquee	25.3 ± 1.3a	45.7 ± 1.2a	30.3 ± 1.0a
Tamora	25.3 ± 1.7a	44.9 ± 1.6ab	31.6 ± 1.1a

*Means in the same column followed by the same letter are not significantly different ($p < 0.05$) as determined by Least Squares Means

Table 3.2 SAS output (ANOVA) for protein and starch concentrations in, and amylose concentrations in starch from, ten pea genotypes.

	Covariance Parameter Estimates		
Covariance Parameter	Protein	Starch	Amylose
Loc[a]	0.7914	0.4644	0.0000
Year	0.3066	0.4311	0.0000
Rep(Loc*Year)	0.0025	0.0000	0.0000
Loc*Year	0.6251	0.4735	0.2184
Loc*Ge[a]	0.1566*	0.0000	0.1247
Year*Ge	0.1514	0.0000	0.1383
Loc*Year*Ge	0.0694	0.6615*	0.0117
Residual	0.3643**	1.1410**	0.8067**

Type 3 Tests of Fixed Effects (Genotype)

Analysis	NumDF[b]	DenDF[c]	F-Value	Pr>F
Protein	9	14.3	7.70	0.0004**
Starch	9	63	3.36	0.0020*
Amylose	9	12.1	2.17	0.1048

* $p < 0.05$; ** $p < 0.001$
[a] where Loc = location and Ge = genotype
[b] NumDF = numerator degrees of freedom
[c] DenDF = denominator degrees of freedom

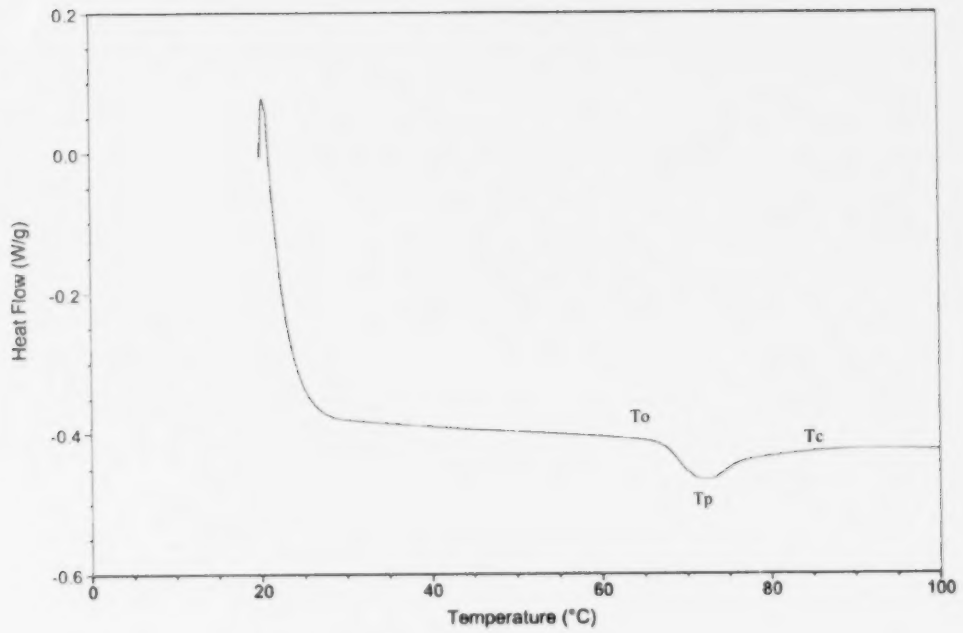

Figure 3.1 Example of a Differential Scanning Calorimetry (DSC) scan of pea starch where To is onset temperature of gelatinization, Tp is peak temperature of gelatinization and Tc is completion temperature of gelatinization.

Table 3.3 Mean values for onset temperature (To), peak temperature (Tp) and completion temperature (Tc) of gelatinization, endothermic enthalpy (ΔH) and the difference between Tc and To (Tc-To) for starch from ten pea genotypes grown in four locations in each of 2006 and 2007.*

Genotype	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	Tc-To(°C)
Bluebird	63.2 ± 2.2a	68.9 ± 1.6a	87.0 ± 2.4a	14.4 ± 1.3a	23.8 ± 2.4a
CanStar	64.1 ± 2.2a	69.7 ± 1.7a	87.9 ± 2.6a	14.3 ± 1.3a	23.8 ± 2.6a
CDC Striker	63.7 ± 2.4a	69.1 ± 1.7a	87.2 ± 3.0a	14.3 ± 1.3a	23.5 ± 3.0a
CDC Tucker	63.5 ± 3.0a	69.0 ± 2.2a	88.0 ± 3.4a	14.8 ± 1.3a	24.5 ± 3.4a
Cooper	64.0 ± 3.2a	69.3 ± 2.4a	87.2 ± 3.5a	14.1 ± 1.3a	23.2 ± 3.5a
Cutlass	61.7 ± 2.3a	69.2 ± 1.5a	87.4 ± 2.5a	14.4 ± 1.3a	23.9 ± 2.5a
Fusion	63.2 ± 2.7a	68.9 ± 1.8a	87.4 ± 3.0a	14.9 ± 1.3a	24.2 ± 3.0a
Reward	62.8 ± 2.5a	68.6 ± 1.8a	87.5 ± 2.4a	14.9 ± 1.3a	24.7 ± 2.4a
SW Marquee	63.9 ± 2.4a	69.3 ± 1.6a	87.7 ± 2.9a	14.1 ± 1.3a	23.8 ± 2.9a
Tamora	63.8 ± 3.1a	69.2 ± 2.2a	87.8 ± 3.2a	14.3 ± 1.3a	24.0 ± 3.2a

*Means in the same column followed by the same letter are not significantly different ($p < 0.05$) as determined by Least Squares Means

Table 3.4 SAS output (ANOVA) for onset temperature (To), peak temperature (Tp) and completion temperature (Tc) of gelatinization, endothermic enthalpy (ΔH) and the difference between Tc and To (Tc-To) of starch from ten pea genotypes.

Covariance Parameter Estimates					
Covariance Parameter	To	Tp	Tc	ΔH	Tc-To
Location	3.6789	2.1927	0.0633	0.8982	2.5322
Year	1.0690	0.3661	0.0000	0.0280	0.2309
Rep(Loc*Year)[a]	0.0000	0.0000	0.0000	0.0000	0.0000
Loc*Year	2.8967	1.1227	0.0000	0.0081	3.7928
Loc*Ge[a]	0.4578	0.2583*	0.0000	0.1253	0.9688
Year*Ge	0.0000	0.0052	0.0000	0.0383	0.0000
Loc*Year*Ge	2.55E-18	0.0913	0.0000	0.2303	0.0000
Residual	5.2699**	0.2269**	10.7231**	0.6628**	6.2185**

Type 3 Tests of Fixed Effects (Genotype)				
Analysis	NumDF[b]	DenDF[c]	F-Value	Pr>F
To	9	27	1.17	0.3539
Tp	9	20.8	0.94	0.5134
Tc	9	147	1.10	0.3669
ΔH	9	10	0.74	0.6705
Tc-To	9	27	0.81	0.6094

* $p < 0.05$; ** $p < 0.001$

[a] where Loc = location and Ge = genotype

[b] NumDF = numerator degrees of freedom

[c] DenDF = denominator degrees of freedom

but there was a significant location by genotype interaction (Table 3.6). There were significant genotypic differences for PV, TV, CV and FV (Table 3.5). Starch from CanStar exhibited the highest PV, TV and CV at 1350, 1230 and 1990 cP, respectively. CanStar, CDC Striker and Cutlass had similar FVs (2100, 2110 and 2080 cP, respectively). Starches from Bluebird, CDC Tucker, Cooper and Tamora exhibited lower CVs than did those from the other pea genotypes. There was a significant location by year by genotype interaction for PV, CV and FV (Table 3.6).

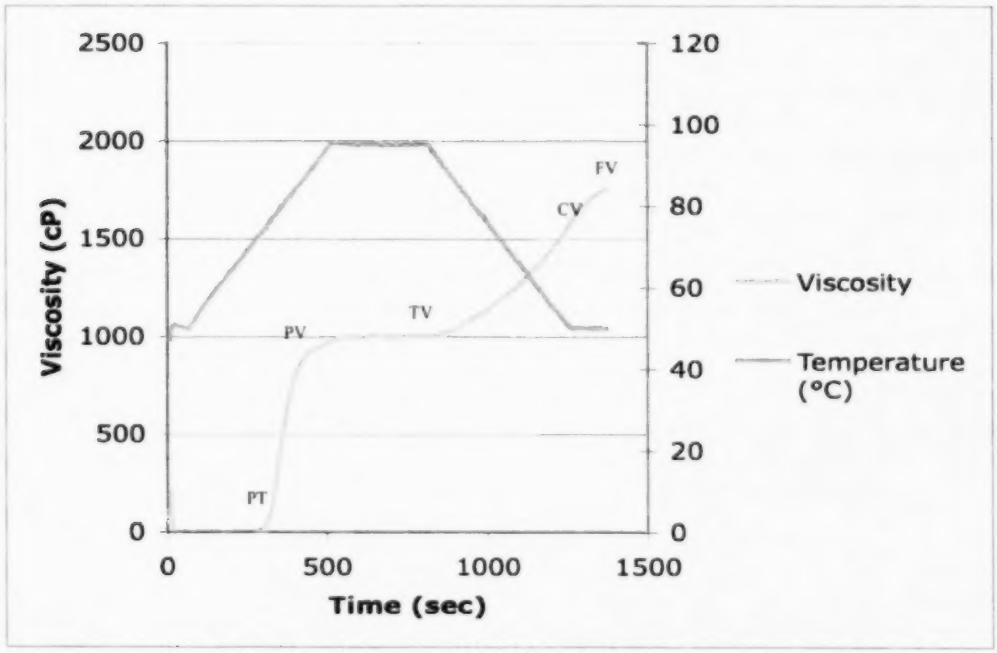

Figure 3.2 Example of a Rapid Visco Analyzer (RVA) graph of pea starch where PT is pasting temperature, PV is pasting viscosity, TV is trough viscosity, CV is cooling viscosity and FV is final viscosity.

Table 3.5 Mean values for pasting temperature (PT), pasting viscosity (PV), trough viscosity (TV), cooling viscosity (CV) and final viscosity (FV) of starch from ten pea genotypes grown in four locations in each of 2006 and 2007.*

Genotype	PT (°C)	PV (cP)	TV (cP)	CV (cP)	FV (cP)
Bluebird	74.8 ± 1.1a	1180 ± 50a	1050 ± 50a	1700 ± 90a	1900 ± 110a
CanStar	75.1 ± 1.3a	1350 ±170b	1230 ± 170b	1990 ± 210b	2100 ± 230b
CDC Striker	74.2 ± 1.0a	1130 ± 90a	1080 ± 80a	1870 ± 150c	2110 ± 190b
CDC Tucker	74.8 ± 1.1a	1170 ± 80a	1100 ± 90a	1790 ± 90ac	1960 ± 120a
Cooper	74.1 ± 1.6a	1140± 110a	1050 ± 60a	1770 ± 170a	1970 ± 200a
Cutlass	74.4 ± 1.2a	1160 ± 110a	1080 ± 60a	1840 ± 140c	2080 ± 180b
Fusion	74.1 ± 1.1a	1140 ± 140a	1070 ± 120a	1810 ± 140c	2030 ± 170a
Reward	74.6 ± 1.2a	1170 ± 80a	1150 ± 90c	1870 ± 160c	2040 ± 230a
SW Marquee	74.4 ± 1.2a	1200 ± 110a	1090 ± 110a	1810 ± 140c	2020 ± 180a
Tamora	74.9 ± 1.5a	1100 ± 70a	1080 ± 70a	1750 ± 110a	1960 ± 140a

*Means in the same column followed by the same letter are not significantly different ($p < 0.05$) as determined by Least Squares Means

Table 3.6 SAS output (ANOVA) for pasting temperature, pasting viscosity, trough viscosity, cooling viscosity and final viscosity of starch from ten pea genotypes.

	Covariance Parameter Estimate				
Covariance Parameter	PT[b]	PV	TV	CV	FV
Loc	0.9430	957.84	343.34	10723	17558
Year	0.1847	0	992.77	0	0
Rep(Loc*Year)[a]	0.0000	55.7040	45.2764	0	0
Loc*Year	0.4375	4427.79	2235.01	808.41	1733.25
Loc*Ge[a]	0.2852*	995.75	0	68.1328	1564.06
Year*Ge	0.0000	0	0	0	0
Loc*Year*Ge	0.0000	4051.47**	3007.69	6970.42**	9913.57**
Residual	0.9886**	1802.07**	2178.35**	3717.83**	4715.16**

Type 3 Tests of Fixed Effects (Genotype)

Analysis	NumDF[c]	DenDF[d]	F-Value	Pr>F
PT	9	27	0.98	0.4759
PV	9	27	6.23	<0.0001**
TV	9	63	6.09	<0.0001**
CV	9	27	5.52	0.0003**
FV	9	27	3.90	0.0028**

* $p < 0.05$; ** $p < 0.001$

[a] where Loc = location and Ge = genotype

[b] where PT = pasting temperature, PV = pasting viscosity, TV = trough viscosity, CV = cooling viscosity and FV = final viscosity

[c] NumDF = numerator degrees of freedom

[d] DenDF = denominator degrees of freedom

3.2 Fababean

Mean values for protein and starch concentrations in eleven genotypes of fababean are presented in Table 3.7. Gloria had the highest protein concentration at 32.4% (dry basis). CDC Fatima, NPZ4-7540 and SSNS-1 contained 30.7, 29.7 and 30.2% protein (dry basis), respectively. CEB04928, NPZ3-7080 and NPZ4-7460 had the lowest protein concentrations at 27.5, 28.2 and 28.3% (dry basis), respectively. CDC Fatima, NPZ4-7540 and SSNS-1 had the lowest starch concentrations at 41.1, 42.8 and 42.5% (dry basis), respectively. Amylose concentrations were similar among starches from the fababean genotypes, ranging from 28.8-30.0%, respectively (Table 3.7). The location by genotype interaction was significant for the concentration of starch in fababean and the concentration of amylose in fababean starch (Table 3.8).

Mean values for the thermal properties of starches isolated from fababean are presented in Table 3.9. An example of a DSC scan for starch isolated from fababean is presented in Figure 3.3. CEB04928 had the lowest To (59.5°C) and Tp (65.8°C). Temperature of completion of gelatinization, ΔH and the difference between Tc and To (Tc-To) were not significantly different for starches from the fababean genotypes. The interaction between location and genotype was significant for To and ΔH (Table 3.10).

Mean values for the pasting properties of fababean starches are presented in Table 3.11. An example of a Rapid Visco Analyzer (RVA) graph for starch isolated from fababean is presented in Figure 3.4. The PT of starches from the eleven genotypes ranged from 71.9-73.3°C (Table 3.11). Disco, Dixie, NPZ3-7080, NPZ4-7460 and NPZ4-7540 had the highest PVs at 1252, 1250, 1280, 1228 and 1247 cP, respectively. There were significant differences among the fababean starches for PV, TV, CV and FV (Table 3.12). The interaction between location and genotype was significant for TV (Table 3.12). Gloria had the lowest TV at 1024 cP (Table 3.11). CDC Fatima, CEB04928, Disco and NPZ4-7540 had the highest CVs at 2010, 2190, 2290, 2130 and 2230 cP, respectively. Gloria and SSNS-1 had the lowest CVs at 1609 and 1780 cP, respectively, and the lowest FVs at 1920 and 2060 cP, respectively.

Table 3.7 Mean values for protein and starch concentrations (%, dry basis) in, and amylose concentrations (%) each of in starch from, eleven fababean genotypes grown in three locations in 2006 and 2007.*

Genotype	Protein	Starch	Amylose
CDC Fatima	30.7 ± 1.5a	41.1 ± 2.3a	29.3 ± 1.4a
CEB04928	27.5± 1.5b	45.3 ± 1.7b	29.6 ± 1.4a
Disco	28.5 ± 1.3c	45.2 ± 3.0b	29.5 ± 1.0a
Dixie	29.7 ± 1.9c	43.8 ± 2.2b	28.8 ± 1.0a
Gloria	32.4 ± 1.4d	44.1 ± 4.8b	28.9 ± 1.0a
NPZ3-7080	28.2 ± 0.9b	43.2 ± 2.6b	29.3 ± 1.0a
NPZ4-7460	28.3 ± 1.4b	45.3 ± 1.4b	29.1 ± 0.9a
NPZ4-7540	29.7 ± 1.4ac	42.8 ± 1.6ab	29.3 ± 1.2a
NPZ5-7530	28.7 ± 1.1c	45.2 ± 1.9b	29.4 ± 1.1a
Snowbird	28.4 ± 1.7c	47.5 ± 3.4c	29.0 ± 0.9a
SSNS-1	30.2 ± 0.8a	42.5 ± 2.5a	30.0 ± 1.1a

*Means in the same column followed by the same letter are not significantly different, ($p < 0.05$) as determined by Least Squares Means

Table 3.8 SAS output (ANOVA) for protein and starch concentrations in, and amylose concentrations in starch from, eleven fababean genotypes.

	Covariance Parameter Estimates		
Covariance Parameter	Protein	Starch	Amylose
Loc[a]	1.0908	2.3225	0.5520
Rep(loc)	0.0000	0.1009	0.0000
Loc*Ge[a]	0.1011	2.2478**	0.1772*
Residual	0.7408**	2.5151**	0.4932**

Type 3 Tests of Fixed Effects (Genotype)

Analysis	NumDF[b]	DenDF[c]	F-Value	Pr>F
Protein	10	47.3	22.14	<0.0001**
Starch	10	47.3	5.22	<0.0001**
Amylose	10	47.3	1.46	0.1863

* $p < 0.05$; ** $p < 0.001$

[a] where Loc = location and Ge = genotype

[b] NumDF = numerator degrees of freedom

[c] DenDF = denominator degrees of freedom

Table 3.9 Mean values for onset temperature (To), peak temperature (Tp) and completion temperature (Tc) of gelatinization, endothermic enthalpy (ΔH) and the difference between Tc and To (Tc-To) of starch from eleven fababean genotypes grown in three locations in each of 2006 and 2007.*

Genotype	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	Tc-To(°C)
CDC Fatima	61.3 ± 2.6a	67.2 ± 2.6a	87.2 ± 1.0a	16.7 ± 1.7a	25.9 ± 2.3a
CEB04928	59.5 ± 2.6b	65.8 ± 2.8b	86.6 ± 1.1a	17.1 ± 1.8a	27.1 ± 1.9a
Disco	60.8 ± 2.1a	66.9 ± 2.3ac	86.8 ± 1.3a	18.0 ± 1.7a	26.0 ± 1.5a
Dixie	61.7 ± 2.7a	67.6 ± 2.5ac	86.8 ± 1.1a	16.9 ± 1.8a	25.1 ± 2.5a
Gloria	61.0 ± 3.0a	66.9 ± 2.9ac	87.1 ± 1.0a	16.7 ± 2.0a	26.1 ± 2.3a
NPZ3-7080	60.9 ± 3.1a	67.1 ± 2.7ac	87.5 ± 1.3a	17.0 ± 1.9a	26.6 ± 2.6a
NPZ4-7460	60.8 ± 2.4a	66.9 ± 2.5ac	86.9 ± 1.6a	16.7 ± 1.8a	26.1 ± 2.1a
NPZ4-7540	61.2 ± 3.1a	66.6 ± 2.7c	86.7 ± 1.3a	17.1 ± 1.7a	25.5 ± 2.5a
NPZ5-7530	60.8 ± 2.6a	66.7 ± 2.6ac	86.6 ± 1.5a	16.9 ± 1.6a	25.8 ± 2.0a
Snowbird	61.4 ± 2.8a	66.9 ± 2.6ac	87.1 ± 1.3a	16.7 ± 1.5a	25.7 ± 2.0a
SSNS-1	61.2 ± 2.5a	66.8 ± 2.5ac	86.8 ± 1.5a	16.8 ± 1.3a	25.7 ± 1.8a

*Means in the same column followed by the same letter are not significantly different ($p < 0.05$) as determined by Least Squares Means

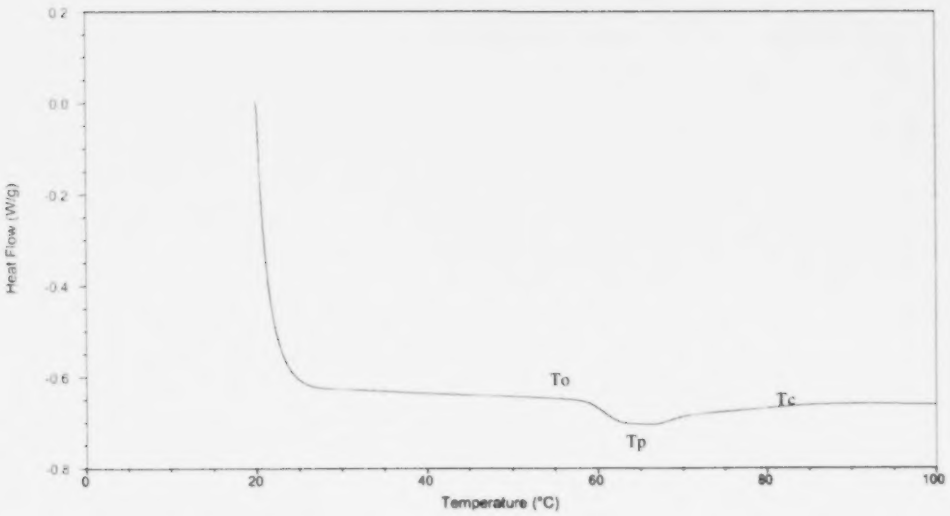

Figure 3.3 Example of a Differential Scanning Calorimetry (DSC) scan of fababean starch where To is onset temperature of gelatinization, Tp is peak temperature of gelatinization and Tc is completion temperature of gelatinization.

Table 3.10 SAS output (ANOVA) for onset temperature (To), peak temperature (Tp) and completion temperature (Tc) of gelatinization, endothermic enthalpy (ΔH) and the difference between Tc and To (Tc-To) of starch from eleven fababean genotypes.

Covariance Parameter Estimates					
Covariance Parameter	To	Tp	Tc	ΔH	Tc-To
Loc[a]	6.6118	6.3712	0.7105	1.9388	3.1345
Rep(loc)	0	0.0081	0.0504	0.0442	0.0275
Loc*Ge[a]	0.1972**	0.0794	0	0.4460*	0.2803
Residual	0.2431**	0.2520**	0.9248**	0.4905**	1.2541**

Type 3 Tests of Fixed Effects (Genotype)				
Analysis	NumDF[b]	DenDF[c]	F-Value	Pr>F
To	10	47.2	5.22	<0.0001**
Tp	10	46.6	4.93	<0.0001**
Tc	10	103	1.01	0.4377
ΔH	10	45.4	1.09	0.3902
Tc-To	10	47.2	1.93	0.0642

* $p < 0.05$; ** $p < 0.001$

[a] where Loc = location and Ge = genotype

[b] NumDF = numerator degrees of freedom

[c] DenDF = denominator degrees of freedom

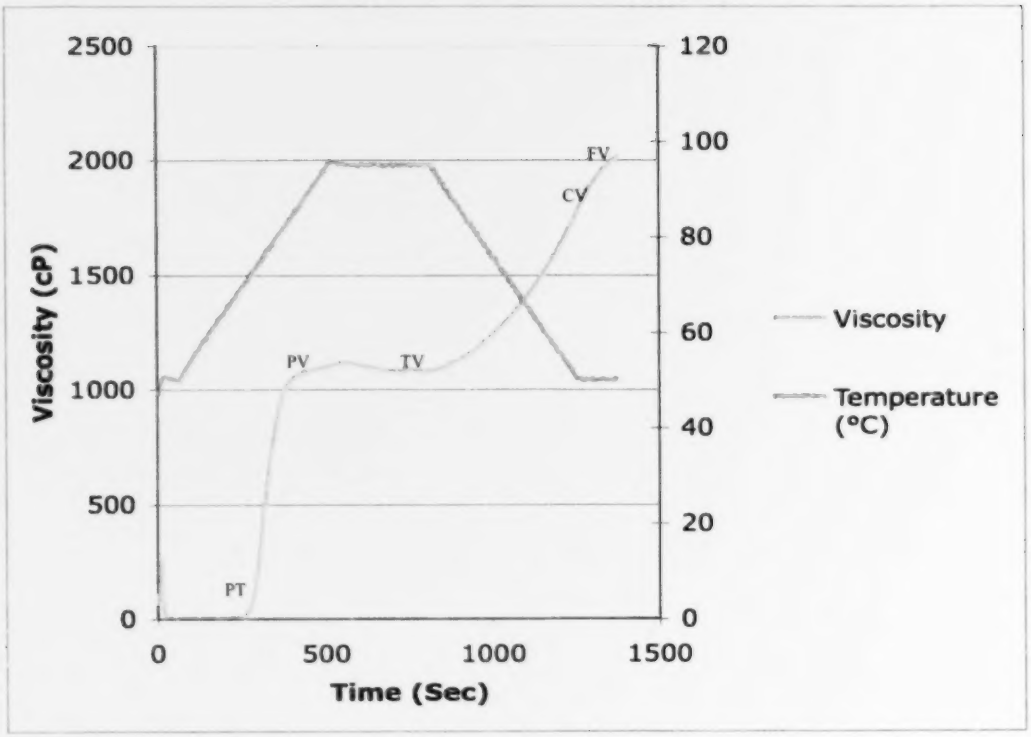

Figure 3.4 Example of a Rapid Visco Analyzer (RVA) graph of fababean starch where PT is pasting temperature, PV is pasting viscosity, TV is trough viscosity, CV is cooling viscosity and FV is final viscosity.

Table 3.11 Mean values for the pasting temperature (PT), pasting viscosity (PV), trough viscosity (TV), cooling viscosity (CV) and final viscosity (FV) of starch isolated from eleven fababean genotypes grown in three locations in each of 2006 and 2007.*

Genotype	PT (°C)	PV (cP)	TV (cP)	CV (cP)	FV (cP)
CDC Fatima	71.9 ± 3.3a	1131 ± 111b	1105 ± 100a	2010 ± 210de	2330 ± 260cd
CEB04928	72.0 ± 3.0a	1162 ± 138b	1160 ± 135ab	2190 ± 350c	2540 ± 430d
Disco	73.0 ± 1.4a	1252 ± 103c	1224 ± 100b	2290 ± 220ac	2660 ± 270bd
Dixie	72.7 ± 1.5a	1250 ± 136c	1210 ± 136b	2060 ± 220de	2350 ± 270cd
Gloria	73.3 ± 2.0a	1037 ± 117a	1024 ± 105c	1690 ± 230b	1920 ± 280a
NPZ3-7080	72.6 ± 1.9a	1280 ± 123c	1223 ± 100b	2400 ± 300a	2810 ± 370b
NPZ4-7460	72.7 ± 1.5a	1228 ± 123c	1227 ± 128b	2130 ± 260ce	2440 ± 320d
NPZ4-7540	72.6 ± 1.7a	1247 ± 96c	1219 ± 99ab	2230 ± 260c	2560 ± 330d
NPZ5-7530	72.5 ± 1.6a	1136 ± 170b	1176 ± 122ab	2090 ± 240e	2450 ± 330d
Snowbird	72.9 ± 2.6a	1170 ± 192b	1153 ± 183ab	1950 ± 440de	2230 ± 520c
SSNS-1	72.8 ± 2.7a	1023 ± 112a	1018 ± 108c	1780 ± 200b	2060 ± 260ac

*Means in the same column followed by the same letter are not significantly different ($p < 0.05$) as determined by Least Squares Means

Table 3.12 SAS output (ANOVA) for pasting temperature, pasting viscosity, trough viscosity, cooling viscosity and final viscosity of starch isolated from eleven fababean genotypes.

Covariance Parameter Estimates

Covariance Parameter	PT[b]	PV	TV	CV	FV
Loc[a]	2.8880	9420.4	9391.8	52409	83059
Rep(Loc)	0	0	219.9	0	0
Loc*Ge[a]	0	1571.5	2322.8*	10567	14509
Residual	1.8327**	7176.5**	3928.8**	16791**	24972**

Type 3 Tests of Fixed Effects (Genotype)

Analysis	NumDF[c]	DenDF[d]	F-Value	Pr>F
PT	10	109	0.97	0.4774
PV	10	46.6	8.26	<0.0001**
TV	10	47	7.86	<0.0001**
CV	10	46.4	12.76	<0.0001**
FV	10	46.5	13.33	<0.0001**

* $p < 0.05$; ** $p < 0.001$

[a] where Loc = location and Ge = genotype

[b] where PT = pasting temperature, PV = pasting viscosity, TV = trough viscosity, CV = cooling viscosity and FV = final viscosity

[c] NumDF = numerator degrees of freedom

[d] DenDF = denominator degrees of freedom

4.0 Discussion

The pea genotypes were lower in protein than were the fababean genotypes (24.1-27.1% versus 27.5-32.4%). The fababean genotypes exhibited a broader range of starch concentrations than did the pea genotypes (41.1-47.5% versus 44.0-46.2%). Typically, pea and fababean starches contain intermediate concentrations of amylose (33-35%) (Biliaderis et al. 1979). Results from this study revealed that the amylose concentrations in pea and fababean starch ranged from 29.8-31.5% and 28.8-30.0%, respectively. Compared to starches from the pea genotypes, starches from the fababean genotypes had lower To (59.5-61.7°C vs. 61.7-64.0°C) and Tp (65.8-67.6°C vs. 68.6-69.7°C) values, but higher ΔH (16.7-18.0 J/g vs. 14.1-14.9 J/g) values. The pasting temperatures of starches from the pea genotypes ranged from 74.1-75.1°C, and those of starches from the fababean genotypes ranged from 71.9-73.3°C.

4.1 Pea

Wang and Daun (2004a) reported that the pea genotypes Alfetta, Carneval, Keoma and Majoret had mean protein concentrations of 22.6, 22.7, 24.0 and 24.0% (db), respectively. Davydova et al. (1995) tested five pea genotypes and reported a range in protein concentration of 22-27% (db). The range in protein concentration of pea in the present study, 24.2-27.1% (db), was similar to those reported previously. Wang and Daun (2004a) reported concentrations of starch from four pea genotypes that ranged from 41.6-47.5% (db), similar to the starch concentrations in the ten pea genotypes tested in the current study, 44.0-46.2% (db).

Ratnayake et al. (2001) reported that starches from the pea genotypes Carneval, Carrera, Grande and Keoma, when grown at the Morden Research Center, Morden, MB, had total amylose concentrations of 48.8-49.6%, as determined by iodine binding after removal of free and bound lipids. Wang and Daun (2004b) reported amylose concentrations of pea starch ranging from 20.7-33.7%. Biliaderis et al. (1979) and Gujska et al. (1994) reported that pea starch contained 33-35% and 34.2% amylose, respectively. Values for amylose concentrations in pea starches from the current study were lower than those of

Biliaderis et al. (1979), Gujska et al. (1994) and Ratnayake et al. (2001), but higher than those of Wang and Daun (2004b).

Ratnayake et al. (2001) reported that the To of pea starches ranged from 61.0-61.4°C, Tp from 66.8-67.5°C and Tc from 75.0-76.0°C. For starches from the ten pea genotypes in the current study, To ranged from 61.7-64.1°C, Tp ranged from 68.6-69.7°C and Tc ranged from 87.0-88.0°C. Davydova et al. (1995) reported that ΔHs for starch from five pea genotypes ranged from 14.1-17.0 J/g. Results for ΔH from the current study fell within this range (14.1-14.9 J/g). Noda et al (1998) reported that lower To, Tp, Tc and ΔH were indicative of more short amylopectin chains. Davydova et al. (1995) reported that pea starches possessing more B-polymorphs than A-polymorphs also had higher ΔHs, which might reflect differences in the packing of B- and A-polymorphs. Pea starches with higher gelatinization temperatures tend to be those containing more A-polymorphs than B-polymorphs (Ratnayake et al. 2002).

Ratnayake et al. (2001) reported pasting temperatures for Carneval, Carrera, Grande and Keoma ranging from 79.0-79.5°C. Gujska et al. (1994) reported a pasting temperature of 73.0°C for pea starch. The ten genotypes in the current study exhibited pasting temperatures ranging from 74.1-75.1°C. Differences between the results of Ratnayake et al (2001) and those obtained in this study may be attributable to analytical methodology (Brabender viscoamylograph with a starch slurry concentration of 9% w/v and pH 5.5 versus RVA with a starch slurry concentration of 8% w/v and pH 7.0), since increasing the starch concentration for pasting analysis increases pasting temperature and the viscosities at 95°C, after the 95°C hold and at 50°C (Abbas et al. 1986). Genotypic differences in the pasting characteristics of pea starch were detected by Ratnayake et al. (2001) and Davydova et al. (1995) and in the current study.

The current study supports the conclusion of Ratnayake et al. (2001) that differences in starch behaviour exist among genotypes of pea grown in the same location. However, the variation in starch behaviour among pea genotypes, compared to differences that exist among cereal starches or other legume starches, was quite small. For example, Wootton and Mahdar (1993) studied twenty-one

genotypes of Australian wheat and found large variability in thermal and pasting characteristics. The peak viscosities of the twenty-one wheat flours ranged from 280-768 Amylograph units, ΔH ranged from 4.6-13.8 J/g, To ranged from 46-53°C, Tp ranged from 57-62°C, and Tc ranged from 64-78°C. The pasting viscosities of the starches isolated from pea ranged from 1100-1350 cP, ΔH ranged from 14.1-14.9 J/g, To ranged from 61.7-64.0°C, Tp ranged from 68.6-69.3°C and Tc ranged from 87.0-88.0°C.

4.2 Fababean

Hill-Cottingham (1983) reported concentrations of protein and starch in fababean of 32.5% and 41.4% (db), respectively (corresponding values from the current study were 27.5-32.4% and 41.1-47.5%, respectively). The amylose concentrations reported in this study (28.8-30.0%) were slightly lower than those reported by Biliaderis et al. (1979) (33-35%), but higher than that reported by Naivikul and D'Appolonia (1979) (24.0%). Naivikul and D'Appolonia (1979) determined amylose concentration by an iodine binding method, which measures apparent amylose rather than total amylose due to the inability of the iodine to displace lipids bound to amylose, hence the lower value in their study.

Biliaderis et al. (1979) reported To, Tp and Tc of fababean starch to be 56, 65 and 83°C, respectively. Values for To (59.5-61.7°C) and Tc (86.6-87.5°C) from the current study were higher than those reported by Biliaderis et al. (1979). However, values for Tp in this study (65.8-67.1°C) were similar to that reported by Biliaderis et al. (1979). Biliaderis et al. (1979) reported a value of 13.75 J/g for ΔH, which is lower than those determined in the current study (16.7-18.0 J/g).

Naivikul and D'Appolonia (1979) reported the pasting temperature of fababean starch to be 66.0°C, whereas Biliaderis et al. (1979) reported a pasting temperature for fababean starch of 72°C. Values for pasting temperature obtained in this study (72.0-73.3°C) were slightly higher than, or similar to, results reported previously.

As was the case for starches isolated from the pea genotypes (Section 4.1), variations in the thermal and pasting characteristics among starches from the fababean genotypes were small compared to those observed in starches from twenty-one Australian wheat genotypes (Wootton and Mahdar 1993).

5.0 Summary and Conclusions

The effects of genotype and environment, and their interaction, on the concentrations of starch and protein in, and the physicochemical properties of starch from, pea and fababean were investigated. Samples of ten pea genotypes were obtained from material grown in four locations [Elrose, Hodgeville, Melfort, Saskatoon (Sutherland)] in each of 2006 and 2007. Samples of eleven fababean genotypes were obtained from material grown in three locations in 2006 [Melfort, Saskatoon (Preston), Saskatoon (SPG)] and 2007 [Outlook, Saskatoon (Preston), Roblin]. The pea and fababean samples were analyzed for their starch and protein concentrations. Additionally, starches isolated from the pea and fababean samples were analyzed for their amylose concentrations and their thermal and pasting characteristics.

Significant genotypic differences in the concentrations of starch and protein in pea and fababean were observed. On a dry weight basis, pea and fababean contained 44.1-46.2% and 41.1-47.5% starch and 24.2-27.5% and 27.5-32.4% protein, respectively. In addition, genotypic differences in the onset temperature (To) and peak temperature (Tp) of gelatinization of fababean starch, and in the pasting, trough, cooling and final viscosities of pea starch and fababean starch, were observed. Significant two-way interactions (location x genotype) were observed for the concentration of starch in fababean and the amylose concentration in, and the To, endothermic enthalpy of gelatinization (ΔH) and trough viscosity of, fababean starch. Significant three-way interactions (location x year x genotype) were observed for the concentration of starch in pea and the pasting, trough, cooling and final viscosities of pea starch.

A primary objective of this study was to provide information to pulse breeders and processors on the effects of genotype and the environment, and their interaction, on the starch and protein contents of, and the functionality of starch from, pea and fababean. On the basis of the results obtained for the genotypes and environments included in this study, it may be concluded that:

1. The effect of genotype on the concentrations of starch and protein in pea and fababean was sufficient to be of practical significance to end-users.
2. Environment did not have a significant effect on the concentrations of starch and protein in pea and fababean. This was in marked contrast to the results of Reichert and MacKenzie (1982) who reported that the concentrations of starch and protein in 198 Saskatchewan producers' samples of a single genotype of pea ranged from 49.7-59.8% and 13.3-27.1% (dry, dehulled seed basis), respectively. The concentration of protein in western Canadian pea (green and yellow) in 2008 ranged from 18.7-29.1%, with an average of 23.2%. The everage in 2007 was 24.5% (CGC 2009). These apparent discrepancies were likely due to the relatively controlled environments from which the samples used in this study were obtained.
3. In keeping with the observations of Ratnayake et al. (2001), genotype and environment had minor effects on the concentration of amylose in, and the physicochemical characteristics of, starch from pea and fababean. These effects would not be of practical significance.
4. The significant interactions detected – location by genotype for protein concentration in pea and starch concentration in fababean, and location by year by genotype for starch concentration in pea – would complicate the manipulation through breeding of the concentrations of protein and starch in pea and fababean.

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